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THE BEHAVIOR OF LIVE FROG EGGS AND FROG LARVAE IN DISTILLED WATER

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ABSTRACT: The factors in survival and development of frog eggs and larvae in distilled water are analyzed, oxygen in the water and nutrition being found to be decisive factors. Experiments with frog eggs and larvae in distilled and common water are reported.

Are frog eggs and frog larvae capable of living and developing in distilled water? On the basis of experiments undertaken in the Spring of 1915, on which I shall report in more detail below, I can answer the question posed herein in the affirmative: both the eggs and the larvae of frogs are capable of living and of developing fully in distilled water, without any difficulty. /604*

At the beginning of my experiments I tried to transfer into distilled water tadpoles which have developed from eggs in the common and very calcareous tap water of our city, Prague.[2] The first experiments failed: the tadpoles transferred into distilled water lived for a maximum of 24 to 48 hours, then they perished under the symptoms of decomposition. I had the same result when I attempted to get the tadpoles gradually used to the distilled water by slowly adding each day a part of distilled water to the common tap water. The tadpoles perished also in these experiments as soon as the dilution had attained a higher degree. I thought then that it will be entirely impossible to keep /605
frog larvae alive in distilled water. Since their death was always accompanied

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[2] The composition of this water (according to official analysis) is the following: in every 1,000 parts of water there are 13.95 mg of KCl, 12.75 mg of Na_2SO_4, 8.90 mg of K_2SO_4, 57.61 mg of $CaSO_4 \cdot H_2O$, 13.36 mg of $Ca(NO_3)_2$, 167.05 mg of $CaCO_3$, 20.96 mg of $MgCO_3$, 9.97 mg of SiO_2 and 0.38 mg of Fe_2O_3.

* Numbers in the margin indicate pagination in the foreign text.

by decomposition that was especially evident on their tails, I explained the negative results by saying that the distilled water, by being a hypotonic solution in comparison with the hitherto accustomed life medium of the tadpoles, causes an osmotic damage in them.

It is well known that amphibians are among the animals whose internal osmotic pressure (i.e. that of the blood) is entirely independent from the environment (see Hedin, 1915, page 49). On the other hand, however, we know that frogs' skin is permeable to water, as shown by Overton's experiments (quoted in Hedin, 1915, page 50). When a frog is placed in a saline solution whose osmotic pressure is greater than that of the frog's blood, the frog's weight will decrease, which is to be attributed to loss of water. In contrast, in weak saline solutions or in distilled water, if their cloaca is closed by the artificial evacuation of the urinary bladder, the frogs will gain weight, and that because of the accumulation of osmotically absorbed water which, under normal conditions, is removed through secretion through the kidneys.

Considering these conditions it could have been perhaps assumed that in my experiments the tadpoles failed to secrete the osmotically absorbed water perhaps because of the incomplete development of the kidney, and that the water thus remained accumulated in the body and caused the animal's death.

Then it occurred to me to aerate the distilled water before putting the tadpoles in it; and indeed: already the first experiment in which the distilled water was aerated for 48 hours, turned out positive. The tadpoles did not perish in the distilled water but lived on, although the water was not aerated any more thereafter. It is clear therefore that in the earlier experiments the death of the tadpoles in distilled water was due to lack of oxygen. The lack of oxygen is explained by the fact that because of the use of great amounts of distilled water in my experiments always fresh distilled water was being used.

It is not possible, however, to consider the lack of oxygen as the /606
only cause of the tadpoles' death. Later on, namely, I observed on several occasions that frog larvae died even in previously aerated distilled water, and, vice versa, I also found that tadpoles sometimes remained alive even in earlier not aerated distilled water. Therefore, in the case of the death of frog larvae in distilled water some other circumstances must also be taken into account. And this pertains not only to distilled water but also to common tap water. In the course of my experiments with frog larvae it often happened that a few hours after changing the water all tadpoles perished. I must note that we paid strict attention in having the added water to be of the same temperature as that of the used water. It was therefore evident - and I believe that on the basis of my experiences I have the right to draw this conclusion - that the cause of the death of the tadpoles placed in distilled water was to be sought not in the water as such, i.e., in its differences (chemical, salinity, physico-osmotic conditions) as compared with common sweet water, but in other secondary conditions which appear also while using common water, and, vice versa, may be absent when using distilled water. This may be derived from the following experiments carried out on several occasions.

When, for the purposes of a new experiment, I had transferred one part of some frog larvae, developed in common tap water, into previously aerated distilled water and the other part into common fresh tap water, those in the distilled water remained alive, while those in the common tap water perished.

One of the most important secondary conditions in the destruction of the tadpoles placed in distilled water during the first experiment is to be sought certainly in the lack of oxygen in the distilled water. If this is prevented by preliminary aerating, then distilled water becomes much more suitable for frog larvae. And vice versa, if by boiling I artificially removed the air from the common tap water, then this also became lethal for frog larvae. But, as I have already stated, the lack of oxygen is neither the only nor the decisive cause of the dying of the frog larvae in unaerated distilled water: namely, as I have mentioned, I could observe on several /607
occasions that tadpoles placed in oxygen-poor distilled water remained alive, while they perished in oxygen-rich tap water. Since, however, the distilled water's oxygen content has proved to be a sufficiently significant factor, I have used in all my subsequent experiments distilled water previously aerated for 48 hours; I kept the frog larvae of the control series in common Prague tap water.

These experiments have shown that frog larvae, in any state of their development, indeed already from the time on when they can be taken from the jelly-like shell of their eggs, may be transferred into distilled water, wherein they continue to live and grow further. Therefore, transfer into distilled water is possible at any state, without damaging it in its vitality in any manner at all. In addition to the seven experiments to be discussed later in more exact details (see also the attached extract from the records of tests VII, VIII, XIV, X, XXI, XXII, and XV), in which I observed for several weeks the development of frog larvae placed into distilled water in various stages of growth. I have convinced myself of this through a special experiment in which each day, beginning from the fifth day after the laying of the egg at which time the moving embryos capable of being removed from their jelly-like shells without damage are already developed, I have transferred some (7 to 10) embryos into (previously aerated) distilled water. Their development was followed up to the state in which the gills after their full development begin to recede, which occurred on the sixth day after the start of the experiment. In all these series the frog larvae (except for a few which may have been damaged during transfer and perished a few hours later) remained alive and developed at the same rate as those in the common tap water.

Distilled water is also a favorable medium for the development of frog eggs. In all six experiments made for this purpose the eggs, after being placed in distilled water, developed without trouble at the same rate as those in common tap water. Unfortunately I succeeded in only three of these /608
experiments to bring the tadpoles to full development (see extract from the records of tests III, IV, and XXV); in the others all tadpoles perished after the changing of the water - tap water in one case and distilled water in the other two. As I have said, I used in these experiments also previously aerated

distilled water which was changed daily, just as the common tap water of the control series. I could find only one single difference between the eggs developed in common tap water and those developed in distilled water: the embryos of the latter were larger than those of the former. This difference was obvious up until the recession of the gills; but later it disappeared almost entirely and frog larvae developing in distilled water were almost the same as those in common tap water, down to small differences in size (see measurement data attached to test record in the extract; tests III, IV, and XXV). The difference perceivable during the embryo development and the first larval period, which was merely that of size and not of the kind of development, is entirely understandable if we consider that according to Davenport's data at this time the growth of the embryos and larvae takes place only through the intake of water. This, however, because of its osmotic conditions, is much easier in distilled water than in the very calcareous and also quite saline Prague tap water I used.

This was probably a phenomenon similar or analogous to the one in Child's (1907) experiments who has found that in the case of *Tubularia* the extension process which drives the grown hydroid from the perisarc is faster in diluted sea water and also that the hydroids grow to a larger-than-normal length. Peebles (1908) has also observed the faster growing of *Tubularia* in diluted sea water; however, this authoress states that neither in her case nor in Child's do we deal with a direct effect of diluted sea water as a hypotonic medium, but that this effect is an indirect one. The effect functions by providing the dilution of growth-retarding organic matter in sea water. Peebles shows this by calling attention to the circumstance that such /609
an acceleration takes place also in artificial sea water (according to Herbst) which is free of any organic matter. But if we consider that 1) the dilution of any organic matter could not be as great as to eliminate the retarding effect, which is anyway questionable, and 2) that - as Driesch (1909, page 45) has pointed out - Herbst's synthetic sea water is hypotonic in comparison with regular sea water, then we see that both in Child's case and in Peebles' it was the same phenomenon, i.e., the direct diluting effect, and that, just as in my experiments on tadpoles, growth took place in consequence of lesser water intake from the hypotonic medium.

The difference in size between frog embryos and frog larvae developing in common and distilled water occurred only if they were not fed. But as soon as the frog larvae began to take nourishment - I began to feed the tadpoles in all my experiments upon the recession of the gills - these differences disappeared almost entirely. In my opinion, therefore, since at this time the kidneys may have begun to function and have evacuated the excess water - whose presence was proven also by the larger dimension of embryos developing in distilled water - by secretion in the urine (in this matter see the significance of the kidneys in the regulation of osmotic conditions in the bodies of amphibians, as mentioned above; see Hedin, 1915, pp. 50-51).

All in all I have raised ten series of frog larvae in distilled water and with each a control series in common tap water. With three series the raising began with eggs; in the others already more or less developed tadpoles were placed in distilled water. (See details of the conduct of these experiments in the attached extracts from the test record). We raised the frog larvae in small glass aquaria or in round glass vessels in which we changed the water daily. I used un-aerated tap water, but, as I have said above, I aerated the distilled water for at least 48 hours before using it. The culture vessels were not aerated further, since through the daily exchange of water the air became sufficiently renewed. /610

Although in neither one of my experiments have I brought the tadpoles up to full metamorphosis, so long as I could follow them they have all shown full vitality and normal development process, so that the metamorphosis could have been attained had it been necessary for my purposes. Thus, there can be no doubt about the living and growing ability of frog eggs and larvae in distilled water.

Albeit the development of frog larvae in distilled water was, on the whole, normal and identical with that in common tap water, I could observe two differences.

One, I have already mentioned above, and it concerns the larger dimensions of the embryos developed from eggs kept in distilled water, which, as indicated, can be explained by the easier penetration of distilled water, as a hypotonic medium in comparison with common tap water, into the developing embryos and larvae in these early stages.

The second difference is the effect of distilled water on the development of tadpoles in the cases of pure meat diet and of mixed diet. Having no idea of the possible outcome, in some experiments I offered the tadpoles pure frog meat, in others I added also various thin algae in the water. I directly observed that the tadpoles fed on both - I have seen the tadpoles eating both meat and algae, and, as a secondary proof, their excrement was green from chlorophil. In these experiments I have observed that although under mixed diet the rate of growth and development of tadpoles placed in distilled water was the same as that of those in common tap water, the development of larvae receiving pure meat diet was considerably slower in distilled water than in common tap water.

The strongest, and consequently the most evident, is the difference in size. In this respect I refer to the attached extract from the test records /611
in which more detailed data on the course of each experiment may be found. Here is a tabulation of the average sizes of frog larvae in the individual experiments:

Experiment No.	Experiments with Mixed Diet — Average Size of Frog Larvae from		Experiment No.	Experiments with Pure Meat Diet — Average Size of Frog Larvae from	
	Common Tap Water	Distilled Water		Common Tap Water	Distilled Water
III	16.04 mm	17,29 mm	VII	28,73 mm	19,88 mm
XXI	20,14 mm	20.42 mm	IV	15,60 mm	12,21 mm
XXII	25,00 mm	25,78 mm	VIII	11,94 mm	9,04 mm
XXV	9,90 mm	9,37 mm	XIV	11,27 mm	8,57 mm
XV	30,28 mm	31,15 mm	X	15,5 mm	10,77 mm

Tr. Note: Commas indicate decimal points.

The differences can be clearly seen in these figures. The results of the individual measurements given in the extracts from the test record show that this growth retardation has taken place in each individual frog larva, and that it was a general phenomenon. It is interesting that the appearance of this retardation in tadpoles developed in common tap water and placed in distilled water only later, was faster than in tadpoles hatched and grown in distilled water. As evidence, I offer the following: in experiment IV, in which the eggs themselves have developed in distilled water, the difference of size betweeen tadpoles from distilled water and tadpoles from common water became visible only 19 days after the placement of the eggs, i.e., at a time when the frog larvae have been gill-less for quite a while. On the other hand, in experiments XV, X, VII, and VIII, where tadpoles developed in common water have been trasnferred into distilled water, these differences could be recognized already after 3, 7, 4, and 7 days, respectively. Whether this difference in the speed of dimensional decrease of the tadpoles can be explained by saying that the frog larvae growing in distilled water since their egg stage are somewhat larger (see table, above) and therefore the size reduction must take longer in order to drop below the size of the tadpoles in the control series, is questionable, since this difference arising from the embryonal development is never as great as to be able to retard /612
by more than 10 days the dimensional drop taking place later.

As already stated, this retardation in tadpoles fed on pure meat and brought up in distilled water was evident not only in growth, i.e., in the size, but also in the development, namely in the process of metamorphosis, which was slower in frog larvae growing in distilled water on a pure meat diet than in frog larvae kept in common tap water. In experiment IV, in which the tadpoles were brought up from the egg stage, the frog larvae of the control series have lost completely their exterior gills already on the 13th day after the placement of the egg, but those brought up in distilled water under identical conditions only after the 15th day. Whoever is familiar with the speed of frog metamorphosis in the stages of gill development and gill recession will know undoubtedly how great a retardation the difference of two days means in the metamorphosis. In experiment X, in which larvae with not yet completely developed exterior gills were placed in distilled water, the recession of the outer gills in the series in distilled water came about only two (2) days later. In experiment VIII as well, in which entirely gill-less larvae were transferred into distilled water, the delay amounted to two (2) days. But this retardation of the metamorphosis was evident not only in the gill development and recession but also in the growth of the whole body, in the proportions, in the shape of the tail, etc., so that there is no doubt that the whole process indicates a retardation of the whole metamorphosis.

I am unable to state at this time what under a pure meat diet the mechanism of this growth- and development-retarding effect of distilled water is, and how this effect comes about. We have though a few data on the effect of the type of food on the development of frog larvae, but these data concern more the problem of the effect of pure vegetable diet than that of pure meat diet. According to Young (1883) frog larvae fed on pure vegetable diet are unable to develop further, their growth is arrested, and they perish. Tornier (1907) also admits that although a vegetable diet is sufficient to keep the /613
tadpoles alive, it is insufficient to bring them to metamorphosis - thus it causes neoteny. But already Born has opposed this idea and has shown that tadpoles of *Rana fusca* can be brought to metamorphosis in a certain number also on a pure vegetable diet, what Babák (1906) could later substantiate on the basis of his experiments. In his experiments Babák has also tested the effect of pure meat diet as compared with pure vegetable and mixed diets, and has demonstrated that frog larvae fed only on meat (frog meat) were more or less smaller than those given pure vegetable or mixed food, and especially if leaves and stalks of *Stellaria media* are used as the vegetable food. However, when he fed the frog larvae on the one hand with pure frog meat and on the other with a mixture of frog meat and synthetic plant proteins ("Pflanzenproteinsubstanz" - plant protein substance made out of pumpkin seeds by Grubler & Co.), then in the latter group he has found growth retardation (whether he found the same retardation in development, i.e., metamorphosis, he does not say).

If we compare these data concerning the effect of different diets on the development of frog larvae, then it becomes clear that they cannot be compared at all with the effect of a pure meat diet and mixed diet on their development as I have found while keeping them in distilled water, and that they are unable to afford a better understanding of the phenomena encountered. Thus the quest for the cause and mechanism of the retarding effect of distilled water on the growth and metamorphosis of frog larvae under pure meat diet is to be left open, and I intend to undertake further experimental research in the matter.

Since the development of frog larvae in distilled water on a mixed diet is just as normal as in common tap water, it is clear that such retarding effect is a secondary one (as the increase in size of the embryos living since the egg stage in distilled water) and is due more the pure meat diet than to the distilled water. Maybe it is a consequence of insufficient removal of various harmful metabolic products generated under pure-meat diet conditions.

If we disregard these secondary collateral phenomena, my experiments /614
doubtlessly show that both the larvae and eggs of frogs are not only able to live untroubled in distilled water but also to develop in it in a completely normal manner. But we can also conclude further that, depending on the conditions, the salts contained in our sweet water, namely Na_2SO_4, K_2SO_4, $CaSO_4$, $Ca(NO_3)_2$, $CaCO_3$, $MgCO_3$, KCl, SiO_3, and some others (e.g. Fe_2O_3) are neither from the chemical nor from the osmotic point of view necessary for the life and development processes of frog eggs and frog larvae. Regarding sweet-water salts the same holds what Loeb (1911) has stated regarding marine salts in the case of *Fundulus*-eggs and young fish; since Loeb has also found that these marine organisms are just as able to grow and develop normally in distilled water as they are in their usual medium.

Prague; July 1915.

Extract From the Experiment Records

This extract pertains only to those ten (10) experiments in which I succeeded in keeping alive the frog larvae of the series brought up in distilled water and in common water, and to follow them in their development. For reasons of brevity, in the following paragraphs I mark the series in distilled water the dist. W.-series, and the control series in common tap water as the K.-series.

Experiment No. III

Eggs laid on 14 April, 1915 have been divided into two (2) series on the same day. One, the K series, was developed in common tap water; the other in distilled water.

April 15: Gastrulation takes place in both series.

April 16: Formation and closing of medullary tubes.

April 17: Development of embryos which are apparently larger in the W series.

April 20: Developed tadpoles, on some of which exterior gills begin to develop. The difference of size between tadpoles in the K and W series becomes stronger. As far as it can be judged on sight, the tadpoles of the W series were 1/5 to 1/4 larger than the tadpoles of the K series.

April 22: All tadpoles in both series have fully developed exterior gills; from this day on the frog larvae are fed mixed nourishment.

April 24: In both series there is a recession of the exterior gills; some larvae are already gill-less. The difference of size between tadpoles of the K and W series has considerably lessened. /615

April 28: Only small differences in size can be found between the tadpoles of the K and W series.

The development of the frog larvae then proceeded in a normal manner: although many perished in both series, I succeeded in bringing up the majority of tadpoles to the stage of development of the rear feet. On 23 May they were placed in 10% formalin for preservation and measured. The data follow:

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	15,5, 14,0. 17,0, 16,5, 17,0, 22,0, 15,0, 13,0, 14.5, 18,5, 16,0, 21,0, 16.0, 20,5, 17,5, 18,0. 16,5, 17,5, 19,0, 26.0, 17,0, 19,0, 21,0, 17.0, 20.0, 20,0	16,0, 17 0, 16,0, 16,0, 25,5, 19,5, 17 0, 21.5, 21,5, 21,5, 21,5, 17,5, 17,0, 15,5, 19.0, 18,5, 23.0, 21,5, 18,0. 24,0, 21,0, 10,0, 16,5, 17,0, 15,0, 18.0, 19,5, 10,5, 10,0, 20,0, 10,0, 19,0
Average Size	16.04 mm	17.29 mm

Tr. Note: Commas indicate decimal points.

Experiment No. IV

Eggs laid on 17 April, 1915 have been divided into two (2) series. One, the K series, was developed in common tap water, the other in distilled water.

April 18: Gastrulation takes place.

April 19: Formation and closing of medullary tubes.

April 20: Development of embryos in both series; those in the W series are larger.

April 24: Developed tadpoles with almost completely developed exterior gills. Tadpoles in the W series are larger. From this day on I fed the tadpoles pure frog meat.

April 27: The exterior gills of the tadpoles of the K series are receding. By contrast the exterior gills of those in the dist. W series are still fully developed, without any trace of recession.

April 29: The exterior gills of all tadpoles in the K series are completely receded; in a few of the dist. W series the recession has begun, but in the majority of tadpoles the gills are still fully in evidence.

April 30: The exterior gills are receded also on the tadpoles of the dist. W series; only on a few remain small vestiges of them. The tadpoles of the dist. W series are not any more larger than those of the K series: they are of the same size.

May 6: The tadpoles of the dist. W series are considerably smaller than those of the K series and also retarded in general development.

On May 8 the tadpoles of both series were placed in 10% formalin for preservation and measured. The data are as follows: /616

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	14,0, 16,0, 15,0, 16,0, 15,5, 16,0, 15,0, 15,5, 16,5, 16,5	12,3, 11,5, 12,0, 13,5, 12,5, 12,0, 11,0, 15,0, 11,5, 10,5, 12,0, 12,0, 13,0
Average Size:	15.6 mm	12.2 mm

Tr. Note: Commas indicate decimal points.

Experiment No. VII

Eggs laid on April 7 developed in common tap water, and as tadpoles grew up with completely receded exterior gills, they were divided into two series, one of which was kept again in common water and the other in distilled water: this was carried out on April 19. As already earlier, the tadpoles of both series were fed pure frog meat even thereafter.

Up to April 24 the development of the tadpoles of both series progressed in the same manner.

On April 25, however, the tadpoles of the dist. W series appeared to be somewhat smaller in comparison with those of the K series, and on April 26 this difference in size became clearly evident; from this time on it increased continuously.

I brought up the tadpoles of both series until the appearance of the rear feet, then, on May 15, I placed them in 10% formalin and measured them. The data are as follows:

	K series	dist. W. series
Size of the Individual Tadpoles in mm:	24,0, 30,0, 30,5, 27.0, 31.0, 27.0, 32,0, 19,0, 33,0, 32,0, 33,0, 28,0, 27,0	17,0, 22.5, 18,5, 18,5, 22,5, 20.5, 21,0, 20,0, 19,8, 18,8, 19,5, 20,0
Average Size:	28.73 mm	19.88 mm

Tr. Note: Commas indicate decimal points.

Experiment No. VIII

Eggs laid on April 21 developed in common tap water, and as tadpoles grew up without exterior gills, they were divided into two series, one of which was kept again in common tap water and the other in distilled water: this was carried out on April 27. Then the tadpoles were fed pure frog meat.

April 28: In both series began the development of exterior gills.

April 29: Full exterior gills evenly developed in both series. /617

April 30: While the recession of the exterior gills began on the tadpoles of the K series, the gills remained in a full state of development on the tadpoles of the dist. W series.

May 2: In the K series the exterior gills have completely disappeared. In the dist. W series the recession is only beginning; also, in this series the tadpoles are smaller than those of the K series.

May 4: The tadpoles of the dist. W series still have some vestiges of the gills, and in comparison with those of the K series, these tadpoles are way behind in size.

May 5: It is only today that the tadpoles of the dist. W series, which are considerably smaller than those of the K series, have attained the stage of complete recession of the exterior gills.

On May 10 the tadpoles of both series were placed into 10% formalin for preservation and measured. The results are:

	K series	dist. W. series
Sizes of the Individual Tadpoles in mm:	10.0, 12,5, 11,0, 12,0, 12,0, 13.0, 12.5, 11.0, 12,5, 11,5, 13,0, 12,0, 13,5, 12,5, 11,5, 11,5, 11,0	9,2, 9,8, 9.0, 8,5, 8,0, 9,3, 9,0, 9,5, 9,3, 8,5, 9,0, 9,3, 8,8, 9,5, 9,0, 9.0
Average Size:	11.94 mm	9.04 mm

Tr. Note: Commas indicate decimal points.

Experiment No. XIV

Eggs laid on 21 April developed in common tap water: as the tadpoles have fully developed their exterior gills, they were divided into two series, one of which was placed into common water and the other into distilled water for further development. This took place on the 5th of May, and the tadpoles were fed on pure frog meat.

May 6: In the K series recession of the exterior gills is taking place; in the dist. W series they are still in full development.

May 7: In the K series the exterior gills have completely disappeared; in the dist. W series gill recession is taking place only on a very few tadpoles. As for size, the tadpoles of the dist. W series are behind those of the K series.

May 9: Although the exterior gills of the tadpoles in the dist. W series have almost completely receded, the difference in size between the tadpoles of this series and those of the K series has considerably increased.

The tadpoles were placed into 10% formalin for preservation and measured. The results are:

/618

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	10.5, 11.0, 11,0, 11 0, 17.7, 12.0, 10.5, 11.7, 9.5, 10.0, 11.2, 11.0, 11.7, 11.5, 12.0, 10.0, 10.5, 12,0, 10,0, 11.0, 10.5, 10.5, 10,0, 11,5, 10,5, 11.0, 11.0, 11,0, 10,5, 11,5, 11.5	7,0, 7,5, 9,0, 8.0, 8.0, 8.0, 9.5, 8,5, 10,0, 8.5, 8,5, 8,0, 7.0, 8.5, 7,0, 7.0, 9.0, 8,7, 8.0, 8,5, 8.0, 9,0, 8,5, 10,0, 7,3, 9,0, 8.5, 7,5
Average Size:	11.27 mm	8.57 mm

Tr. Note: Commas indicate decimal points.

Experiment No. X

Tadpoles developed in cold water from eggs laid on April 21, having reached the initial stage of exterior gills, have been divided into two series, one of which was left in common water and the other transferred into distilled water. This took place on 28 April, and thereafter both series were fed on pure frog meat.

On 29 April the exterior gills reached full development in both series.

May 1: On the tadpoles of the K series the exterior gills are receding, while on those of the dist. W series they remain in a stage of full development.

May 3: In the K series the tadpoles are completely without gills; in the dist. W series, by contrast, their recession is just beginning. Also, the tadpoles of the latter series are smaller than those of the K series.

May 6: The tadpoles are completely without exterior gills in both series, but the difference in size between the tadpoles of the K series and those of the dist. W series has considerably increased.

This difference increased further during the following days. Then as on May 23 the tadpoles preserved in 10% formalin were measured, the measurement produced the following data:

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	14,5, 16,5, 16,0, 14,5, 15,0, 16,3, 15,5, 14,8, 15,0, 16,0, 16,5, 15,5	10,0, 11,0, 8,5, 12,2, 9,0, 10,5, 9,8, 11,2, 9,5, 12,5, 12,5, 11,8, 12,0
Average Size:	15.5 mm	10.77 mm

Tr. Note: Commas indicate decimal points.

Experiment No. XXI

Eggs laid on May 1 were allowed to develop in common tap water. As the tadpoles reached the stage of full gill development, on May 14 they were divided into two series: one was left in common tap water, the other was transferred into distilled water. Then the tadpoles of both series were fed /619
on a mixed diet of frog meat and algae.

May 15: The recession of the exterior gills began in both series simultaneously.

May 17: Gill completely recessed in both series.

May 19: In both series the development of the tadpoles goes on normally and evenly, only in the dist. W series the tadpoles appear to be somewhat larger.

On May 30 the tadpoles of both series were placed into 10% formalin for preservation, and measured. The results are as follows:

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	20,5, 21,3, 19,5, 19,2, 18,7, 22,0, 21.5, 21,5, 20.7, 19,5, 20,4, 21,3, 18,5, 18,3, 18,0, 21,3	19,1, 19.5, 23,0, 22.5, 19,9, 21,0, 20,5, 22,5, 21,0, 19,7, 19,0, 18,9, 19,7, 20,3, 20,1, 20,0
Average Size:	20.14 mm	20.42 mm

Tr. Note: Commas indicate decimal points.

Experiment No. XXII

Eggs laid on May 1 were developed in common water, and as the tadpoles growing up on a mixed diet of frog fish and algae had their exterior gills recessed, they were divided into two series, one of which was left in common water, the other transferred into distilled water. Both series continued on a mixed diet.

In both series the development of the tadpoles went on evenly and normally. It was only from May 23 on that the tadpoles of the dist. W series appeared to be somewhat larger than those of the K series.

On June 1 the tadpoles of both series were placed into 10% formalin for preservation and measured. The results are as follows:

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	25,0, 24,0, 24,3, 23.8, 23,5, 26,5, 27.0, 25.5, 24,5, 26,0, 27,0, 26,5, 23,5, 24,0, 24,0	27,3, 26.9, 26,7, 24,3, 25.8, 27,0, 26,0, 25,3, 23,9, 25,7, 26.3, 27,0, 26.8, 25,9, 23,8, 24,5, 25,0, 25,0, 25,9, 26,0, 26,3
Average Size:	25.00 mm	25.78 mm

Tr. Note: Commas indicate decimal points.

Experiment No. XV

Eggs laid on April 28 were brought to development in common tap water. Before their exterior gills began to show I divided the developed tadpoles into two series, one of which developed further on in common water, the others were transferred into distilled water. This took place on May 3. Both series were fed again a mixed diet of frog meat and algae. /620

May 5: The exterior gills are evenly developing in both series.

May 7: The exterior gills are completely developed in both series.

May 8: In both series the recession of the gills begins.

May 10: The gills have completely disappeared in both series, and the tadpoles of the dist. W series appear to be somewhat larger than those of the K series.

In the following days the development of the tadpoles of both series went on in a completely normal and even manner, only the difference in size became somewhat sharper between the tadpoles of the K series and those of the dist. W series.

On 29 May the tadpoles of both series were placed in 10% formalin for preservation and measured. The results are as follows:

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	30.3, 31.5, 32.2, 28,5, 29.2, 27.0, 29.9, 30.0, 30,0, 31.5, 28,0, 29,0, 31.5, 32.0, 32,0, 32,5, 33,0, 29,5, 29.0, 28.9	33.0, 32.5, 32,3, 28,5, 29.7, 29,5, 30,5, 33.0, 33.5, 32.0, 32.2, 31,8, 32,5, 33,3, 28,0, 29,7, 29,3, 29,5
Average Size:	30.28 mm	31.15 mm

Tr. Note: Commas indicate decimal points.

Experiment No. XXV

Eggs laid on May 20 were cultivated in part in common tap water (K series) and in part in distilled water (dist. W series). In both series the eggs developed in an even and completely normal manner.

May 21: Gastrulation has taken place.

May 22: Formation of embryos which were considerably larger in the dist. W series.

May 25: Developed tadpoles still without gills. The difference in size between the tadpoles of the K series and those of the dist. W series has increased. The tadpoles of the dist. W series are by 1/5 - 1/4 larger than those of the K series. From this day on the tadpoles of both series are given a mixed diet of frog meat and algae.

May 27: The exterior gills developed in both series. The difference in size between the tadpoles of the K series and those of the dist. W series has strongly decreased, but the tadpoles of the dist. W series still remain clearly larger than those of the K series.

May 29: The exterior gills in both series recessed. The difference in size has further decreased, but still quite evident.

On June 5 the tadpoles of both series were placed into 10% formalin for preservation and measured. The results are as follows:

	K series	dist. W series
Sizes of the Individual Tadpoles in mm:	9.0, 10,0, 8,8, 9,5, 9,5, 10,2, 10,5, 9,2, 10,5, 11,2, 11,0, 8,8, 10,0, 10,0, 9,5, 10,3, 9,5, 9,8	10,5, 9,0, 8,7, 11,5, 10,0, 8,5, 9,5, 9,5, 9,0, 9,7, 8,2, 8,7, 9,0, 11,0, 8,0, 8,5, 9,5, 10.0
Average Size:	9.95 mm	9.37 mm

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Tr. Note: Commas indicate decimal points.

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